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Magnifying Ion Mobility Spectrometry – Mass Spectrometry Measurements for Biomolecular Structure Studies

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Abstract

Ion mobility spectrometry – mass spectrometry (IMS-MS) provides information about the structures of gas-phase ions in the form of a collision cross section (CCS) with a neutral buffer gas. Indicating relative ion size, a CCS value alone is of limited utility. Although such information can be used to propose different conformer types, finer details of structure are not captured. The increased accessibility of IMS-MS measurements with commercial instrumentation in recent years has ballooned its usage in combination with separate measurements to provide enhanced data from which greater structural inferences can be drawn. This short review presents recent outstanding developments in scientific research that employs complementary measurements that when combined with IMS-MS data are used to characterize the structures of a wide range of compounds.

Graphical Abstract

Introduction

Ion mobility spectrometry (IMS) experiments have traditionally employed a drift tube with stacked electrostatic rings used to establish a well-defined, constant electric field (E) [1,2]. The drift region is filled with an inert buffer gas where analyte ions are separated due to differences in their overall size and charge. The transit time (or drift time) of an ion in the drift region (t_D) can be related to its collision cross section (Ω) using [3]:

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$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_b T)^{1/2}} \left[\frac{1}{m_I} + \frac{1}{m_B} \right]^{1/2} t_D \frac{E}{L} \frac{760}{P} \frac{T}{273.2} \frac{1}{N} \quad (1).$$

The variables in Equation 1 include: ion charge (ze), the Boltzmann constant (k_b), the reduced masses of the ion and the buffer gas (m_I and m_B), the length of the drift tube (L), the pressure and temperature of the buffer gas (T and P), and the neutral number density at STP (N). Essentially, from Equation 1, biomolecular ion size differentiation becomes apparent because ions of a larger physical size experience a greater number of collisions with the buffer gas in the drift tube thus increasing t_D and ultimately the determined collision cross section (CCS).

Shortly after the introduction of soft ionization techniques for the production of biomolecular ions [4–6], IMS was combined with mass spectrometry (MS) measurements for the study of gas-phase biomolecular ions [7,8]. Such work led to considerations of the type and degree of structural information that could be garnered from IMS-MS measurements. Early studies focused on comparisons of an ion's drift time to that obtained for different ion conformer types often obtained by *in-silico* manipulation of established solution-phase structures [7,9]. This provided a similarity comparison relating the structures of ions to conformer types having different degrees of elongation and compactness. Although this seminal work laid the foundation for the use of IMS-MS in many ion structure studies, the limited structural details afforded by IMSMS became readily apparent.

Early experiments were followed by notable advances in scientific instrumentation development that significantly expanded the reach of IMS-MS for biomolecular structure analysis. These efforts were primarily centered on improving the sensitivity and resolution of the measurement as well as the ability to manipulate ion structure. One of the most important developments with regard to sensitivity was the coupling of the dispersive IMS separation step with a dispersive MS analysis to improve ion utilization in IMS-MS measurements [10,11]. Development with regard to sensitivity also focused on the use of ion trapping devices that allowed the storage of continuously-produced ions for the pulsed mobility measurement [12–14]. Instrumentation development centered on ion focusing and ion extraction was also pursued to improve measurement sensitivity [14–16]. With regard to resolution improvement, early efforts focused on devices that could sustain higher voltages for improved separations [17–19]. Finally, ion structure manipulation was demonstrated early in the form of inducing precursor ion fragmentation or conformer transformation via collisional activation [20–22]. Although many other outstanding instrument advances were accomplished during the early development time period, arguably those highlighted above played a significant role in the commercialization of IMS-MS instruments. Commercialization of such instrumentation resulted in accelerated adoption of IMS techniques for all levels of biomolecular structure analysis.

Recent years have witnessed a significant expansion in the usage of IMS-MS in structure studies where collision cross section information is combined with results either from separate structural measurements or the observation of changes in IMS measurements

resulting from instrument parameter or sample perturbation. The additional data afforded by the new approaches helps to refine details of ion structure. Such experimental accoutrements can be broadly categorized into on-line and off-line approaches. The former category would represent any supplemental approach occurring as part of (or during) the IMS-MS measurement. An example would be the use of ion reactions that occur before, during, or after an ion's mobility measurement. Off-line approaches would include the use of separate measurements performed on or for the same analyte. An example would be the use of spectroscopic techniques to provide additional information about biomolecular structure. Below, a number of recent advances in these different areas is presented. Notably, an exhaustive review is not provided; rather, high impact studies for online and offline approaches are presented in that order.

On-line approaches (OnA): solution and ion source manipulations.

The first area of discussion considers manipulations that are applied to the sample immediately prior to the production of ions or to the ion source that ultimately affect the information obtained from IMS-MS measurements. A recent study describing sample manipulation was performed by El-Baba *et. al* which showed the monitoring of distinct solution conformer populations of ubiquitin during protein unfolding induced by heating the electrospray solution at the needle [23**]. Remarkably, for the two-state cooperative process, multiple conformers for a number of ion charge states are observed along the transition path as shown in Figure 1A. The work challenges the long-held notion that along this pathway, only unstructured species should be encountered. Another study in this category coupled on-line solution-phase hydrogen/deuterium exchange (HDX) with mobility separation and MS analysis [24]. In separate work using temperature control immediately prior to and during the mobility separation, Servage *et. al* discovered that $[2M+14H]^{14+}$ ions could be stabilized by a very limited number of water adducts prior to dissociation into $[M+7H]^{7+}$ ions [25]. Finally, one study showed significant ability to alter gas-phase conformers and distinguish carbohydrate isomers with the addition of different metal cations [26].

OnA: gas-phase reactions.

One area of research that has expanded in recent years uses higher-pressure regions of IMS-MS instrumentation to perform gas-phase hydrogen/deuterium exchange (HDX) reactions. Khakinejad *et. al* recently used IMSHDX and tandem MS (employing electron transfer dissociation – ETD [27]) to more accurately estimate conformer populations comprising mobility features [28]. Here, the modeled reactivities of candidate ion conformers (see Figure 1B) from molecular dynamics (MD) trajectories were used to assign ion conformer contributions to overall conformer type abundance. In separate studies, Rashid *et. al* utilized a dual spray ion source to perform gas-phase HDX in the source region of a traveling wave ion mobility (TWIM) instrument [29]. The approach was used to study the conformations of peptide and protein ions. Others have used gas-phase HDX to distinguish isomeric carbohydrates[30] and other isobaric small molecules including peptides, metabolites, and lipids[31,32] according to differences in ion structure.

Other experimental work has utilized ion-ion reactions in downstream regions of IMS-MS instrumentation. Recently, Laszlo and Bush utilized cation-to-anion-proton-transfer

reactions (CAPTR)[33] in the ion transfer region of a TWIM instrument to monitor the products resulting from the reaction of compact and elongated cytochrome *c* ions [34*]. Product ions were observed to have very similar collision cross sections. In separate studies the approach was used to suggest that the ion charge should be considered carefully when attempting to relate collision cross sections to solution conformers for a number of different proteins (Figure 1C) [35].

OnA: ion activation and dissociation.

The use of collisional activation of biomolecular ions to induce structural transformations or ion fragmentation in a drift tube was first demonstrated more than a decade ago [36,37]. In recent years remarkable work has been conducted to develop the robust tool of collision-induced unfolding (CIU) for structural characterization [38]. Recently Eschweiler *et. al* have demonstrated the usage of CIU in which unfolding pathways of a homologous group of serum albumins were elucidated [39]. These experiments utilized the CIU patterns of protein-ligand species as well as non-covalent protein complexes to aid in the determination of unfolding pathways. Additionally, the high-reproducibility of CIU patterns was used recently to distinguish structural differences in a biosimilar as shown in Figure 2A [40]. In another seminal report, Allison *et. al* presented an experimental scheme involving the use of CIU for proteins and protein-ligand complexes to ascertain the conformational stability imparted by ligand binding (including multiple ligands) [41**]. The approach is robust and may allow for the determination of even limited structure stabilization upon ligand binding.

In addition to high-pressure ion activation, recent research has focused on the use of post-mobility ion activation to achieve ion dissociation. Song *et. al* demonstrated the power of surface-induced dissociation (SID) with IMS separations which provided important information for determining the relative position and orientation of protein subunits for the protein hexamer toyocamycin nitrile hydratase [42]. More recently this combination has been used to study trimeric and tetrameric membrane protein complexes [43]. Collision-induced dissociation (CID) techniques have also been demonstrated recently. Morrison *et. al* examined metalated tetrasaccharides and observed multiple conformers for several species suggesting the presence of multiple coordination sites [44]. Additionally, the ion fragmentation efficiencies of metalated dimers were observed to be dramatically different. Separate studies reported by Harper *et. al* used CID to aid in the deconvolution of ion drift time distributions; extracted drift time distributions for unique fragment ions helped increase the accuracy [45]. Morrison and Clowers also examined metal ion adduction with ultraviolet photodissociation (UVPD) for distinguishing glycan ions that exhibit overlapping drift time distributions [46*]. The improved ability to distinguish ions results from the combination of metal cationization and UVPD which offers two important advantages namely: 1) improved mobility resolution of glycan ions; and, 2) increased fragmentation efficiency and production of diagnostic ions (Figure 2B).

OnA: IMS with ion infrared (IR) spectroscopy.

Within the last decade improvements in laser technology have significantly enabled the analysis of ion structure using spectroscopic techniques [47]. In concert, a number of recent studies have taken advantage of IMS as a means to isolate ion conformers for spectroscopic

characterization. In probing the ion structures of myoglobin and β -lactoglobulin by monitoring the amide-I and amide-II bands, Seo *et. al*/determined their degree of helicity [48]. For low charge states, myoglobin exhibited a high-degree of helicity while β -lactoglobulin ions of the same charge state produced a spectrum indicative of β -sheet character. In a more recent study, ion IR spectroscopy was coupled with IMS-MS techniques to study the degree of β -sheet character of peptide oligomer ions [49**]. For highly dynamic, small-molecule ions, Voronina *et. al*/have performed cold-ion spectroscopy measurements on mobility-selected peptide ions [50]. They determined that a low-mobility dataset feature was comprised of multiple, kinetically-trapped conformers while a higher-mobility dataset feature corresponded to lower-energy conformers. More recent studies have used cryogenic messenger tagging ion spectroscopy with IMS-MS to assign unique spectral bands for isomeric glycosaminoglycan ions [51]. In separate experiments, cryogenic vibrational spectroscopy in combination with mobility separation is shown to distinguish 6 disaccharide isomers including those that are different by a single stereogenic center [52]. Additionally the approach was demonstrated for distinguishing larger species indicating utility for glycomics and glycoproteomics analyses.

Off-line approaches (OffA): IMS and spectroscopy.

Having discussed hybrid methods that are performed online with IMS-MS measurements, it is instructive to consider approaches for which off-line analyses are used to supplement IMS-MS analysis. For purposes of brevity, novel studies are categorized as spectroscopic, other analytical techniques, and structure modeling. Nyon *et. al*/combined nuclear magnetic resonance spectroscopy, x-ray crystallography, and IMS-MS measurements to study conformational dynamics of variant forms of α -antitrypsin [53]. Overall the results show that IMS-MS is a useful tool for monitoring protein stability upon ligand binding and it is also suggested that such combined measurements could be very useful in the pursuit of drug development. In other studies, IMS-MS measurements (Figure 3A) were combined with small angle x-ray scattering (SAXS) spectroscopy to study enzymatic variants and provided structural information that was not typically obtained for ion exchange chromatography isolates [54]. Other experiments combined fluorescence spectroscopy and gas-phase dimer-induced fluorescence self quenching with IMS-MS measurements to study the structures of small peptides and ubiquitin [55]. The new gas-phase approach provides structural information over very short distances that cannot be revealed by other techniques such as FRET. Finally, double electron-electron resonance (DEER) spectroscopy measurements with IMS-MS analyses was used to detail conformation ensemble changes for the 70 kilodalton heat shock protein DnaK that are associated with the binding of different ligands [56].

OffA: other analytical techniques.

A variety of other measurement strategies have been combined with IMS-MS measurements to characterize biomolecular structure. Here a few studies encompassing such measurement diversity are highlighted. In one study, capillary electrophoresis (CE) was combined with IMS-MS to follow the folding transitions of a polyproline peptide [57*]. Overall, as shown in Figure 3B, a number of structural transformations presented by the CE electropherogram correlated with changing gas-phase conformers distinguished by IMS. CE with UV detection has also been coupled with IMS-MS to monitor protein conformer interconversion

[58]. In separate but related studies, Garabedian *et. al* and coworkers used photoacoustic calorimetry analysis (PAC) with trapped ion mobility spectrometry (TIMS)-MS to study structural transformations of i-motif DNA resulting from pH changes [59]. Boschmans *et. al* used IMS-MS with LC-MS/MS experiments for the characterization of small molecules [60]. For one compound, IMS reveals the presence of two protomers suggesting the need for caution in the structural interpretation of LCMS/MS data.

A number of studies have shown that IMS-MS measurements significantly enhance proteomics measurements by increasing the overall dynamic range of the analysis [61,62]. Recently, however, Lossel *et. al* used traditional proteomic approaches to locate protein phosphorylation sites on the kinase coactivator Bora [63]. Multisite phosphorylation could then be associated with conformational changes of Bora assessed by IMS-MS measurements; such transformations were necessary to form the Bora:kinase protein complex. Another example of the use of separate MS measurements is the study of structural transformations of the decarboxylase enzyme Fdc1 in the presence of the FMN cofactor [64**]. Here IMS-MS was used to examine the conformer types formed in the presence and absence of the cofactor; separately, solution-phase HDX with MS confirmed a conformational “tightening” in the presence of the cofactor.

OffA: molecular modeling.

Perhaps due to its early utilization to aid structure analysis, molecular modeling may be considered the quintessential structure analysis method accompanying IMS-MS measurements. The use of molecular modeling to extract structural information from IMS-MS measurements continues to increase. Here we present distinguishing work which features the use of molecular modeling techniques as well as recent advances in computation approaches used in elucidating biomolecular ion structure. Porrini *et. al* reported the use of extensive molecular modeling (density functional theory, semiempirical calculations, and molecular dynamics simulations - MD) to determine the origin of oligonucleotide duplex ion compaction as observed by IMS-MS analysis [65]. Compaction was attributed to increased attractive interactions compared with Coulomb repulsion. Chouinard *et. al* developed a strategy to sample conformational space using high-temperature MD and implicit solvation modeling followed by gas-phase structure optimization to obtain plausible structures for 25-hydroxyvitamin D3 sodiated epimers with cross sections matching experimental values [66*]. Figure 4A shows the hypothetical sodiated structures with matching collision cross sections for two conformer types. Ghassabi Kondalaji *et. al* recently introduced an MD simulation approach that coupled extensive simulated annealing to capture higher-energy and more stable ion structures as well as MD trajectory analysis with conformer clustering to obtain candidate ion structures [67]. The potential structures were later filtered with gas-phase HDX modeling to provide an estimate of the contributions of different conformer types to observed features in IMS-MS datasets [28].

Algorithms that allow the accurate computation of candidate ion CCS values significantly enabled the use of molecular modeling with IMS-MS measurements for biomolecular structure studies [68,69]. Over the last decade, a number of new algorithms have been developed for these calculations including the projected superposition approximation (PSA),

[70] IMoS,[71] and IMPACT[72] methods. Since that time, work has continued to accelerate with regard to algorithm development and testing as well as their application in CCS calculations. Wyttenbach *et. al*/recently incorporated new capabilities into the PSA approach in which the concavity of the molecular surface is more accurately considered in the cross section determination [73]. In other recent work, Shrivastav *et. al* performed a benchmark comparison of IMoS with the gold standard MOBCAL[68,69] software and determined that the same accuracy in collision cross section calculation could be achieved in a much shorter (100 fold) time as shown in Figure 4B [74*]. Studies are also now elucidating the effect of the charge distribution and suggest that careful consideration should be given to charge configuration as it relates to accurate collision cross section determinations [75]. As an example of the power of the new approaches, consider recent work performed by Landreh *et. al*. The researchers employed the IMPACT software to aid in the modeling of the Na⁺/H⁺ exchanger NapA from *Thermus thermophilus* [76]. Results from the gas-phase study suggest that lipid interactions stabilize the native structure. Other studies have used extensive MD simulations with CCS calculation using IMPACT to delineate the structural details of a trimeric chaperone and outer-membrane proteins [77].

Conclusions

With the expanding use of IMS-MS techniques for structure characterization, a significant amount of research is emerging in which IMS-MS measurements alone provide only a portion – albeit significant – of the information used in ion structure characterization. In this brief review, a limited snapshot of diverse ways in which IMSMS can be accessorized by other measurements or approaches to enhance structure determination is presented. This diversity runs the gamut from sample perturbations to ion perturbations to the inclusion of separate, stand-alone measurements. These combinations have significantly expanded the range and quality of biomolecular structure characterization.

The coming years are expected to witness an increased willingness to combine a variety of techniques with IMS-MS measurements in which the latter continue to play a central role in ion structure determination. Perhaps the area that will be most impacted by development and adoption of combined measurements is the study of all levels of biomolecular conformational heterogeneity [78]. Although other analytical techniques are making inroads with regard to characterizing structural heterogeneity [79], challenges remain with regard to the number of separate conformers that can be addressed as well as those exhibiting slight differences along a continuum of related structural types. Additionally, such approaches are of limited utility in the characterization of co-existing building blocks associated with molecular complex conformation establishment. In contrast, IMS-MS analysis lends itself to the observance of these different, co-existing species including those of low abundance. And, with the coupling of complimentary structure analysis methods (e.g., on-line gas-phase HDX as noted above), the ability to distinguish species of increasing structural similarity is further enhanced.

For wide-scale adoption of combined techniques, a number of factors must be overcome. Two prominent factors are the required expertise in disparate scientific fields and the limited visibility of the usage of combined approaches. The former problem can be addressed by

increased collaborative efforts between and among research laboratories. One positive sign of the trend toward increased collaborative efforts has been the remarkable growth in international collaboration efforts [80]. With regard to the second problem, perhaps for many of the combined approaches highlighted above, the biggest factor critical mass as it is related to research dissemination. That is, as more scientists are made aware of the types of analyses that can be performed and their advantages, the greater will be the adoption and adaption of such techniques. As is often the case, overcoming such a barrier can serve as a positive feedback loop for the other barrier (and vice versa). Overall, the usage of existing and emerging structure measurement tools with IMS-MS methods is expected to proliferate markedly over the coming decade.

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References and recommended reading

Highlighted papers of significant interest are denoted as being of *special or **outstanding interest.

1. Stlouis RH, Hill HH: ION MOBILITY SPECTROMETRY IN ANALYTICAL-CHEMISTRY. *Critical Reviews in Analytical Chemistry* 1990, 21:321–355.
2. Revercomb HE, Mason EA: THEORY OF PLASMA CHROMATOGRAPHY GASEOUS ELECTROPHORESIS – REVIEW. *Analytical Chemistry* 1975, 47:970–983.
3. Mason EAM EW: *Transport Properties of Ions in Gases*. New York: Wiley; 1988.
4. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM: ELECTROSPRAY IONIZATION FOR MASS-SPECTROMETRY OF LARGE BIOMOLECULES. *Science* 1989, 246:64–71. [PubMed: 2675315]
5. Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T, Matsuo T: Protein and polymer analyses up to m/z 100 000 by laser ionization time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry* 1988, 2:151–153.
6. Karas M, Hillenkamp F: Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Anal Chem* 1988, 60:2299–2301. [PubMed: 3239801]
7. Clemmer DE, Hudgins RR, Jarrold MF: Naked Protein Conformations: Cytochrome c in the Gas Phase. *Journal of the American Chemical Society* 1995, 117:10141–10142.
8. Wyttenbach T, vonHelden G, Bowers MT: Gas-phase conformation of biological molecules: Bradykinin. *Journal of the American Chemical Society* 1996, 118:8355–8364.
9. Shelimov KB, Clemmer DE, Hudgins RR, Jarrold MF: Protein structure in vacuo: Gas-phase confirmations of BPTI and cytochrome c. *Journal of the American Chemical Society* 1997, 119:2240–2248.
10. Hoaglund CS, Valentine SJ, Sporleder CR, Reilly JP, Clemmer DE: Three-dimensional ion mobility TOFMS analysis of electrosprayed biomolecules. *Analytical Chemistry* 1998, 70:2236–2242. [PubMed: 9624897]
11. Gillig KJ, Ruotolo B, Stone EG, Russell DH, Fuhrer K, Gonin M, Schultz AJ: Coupling high-pressure MALDI with ion mobility/orthogonal time-of flight mass spectrometry. *Analytical Chemistry* 2000, 72:3965–3971. [PubMed: 10994952]
12. Hoaglund CS, Valentine SJ, Clemmer DE: An ion trap interface for ESI-ion mobility experiments. *Analytical Chemistry* 1997, 69:4156–4161.
13. Myung S, Taraszka JA, Lee YJ, Moon MH, Valentine SJ, Koeniger SL, Clemmer DE: Development of high-sensitivity ion trap-IMS-TOF techniques: A high-throughput nano-

LC/IMS/TOF-separation of the *Drosophila melanogaster* proteome. Abstracts of Papers of the American Chemical Society 2003, 226:U112–U112.

14. Tang K, Shvartsburg AA, Lee HN, Prior DC, Buschbach MA, Li FM, Tolmachev AV, Anderson GA, Smith RD: High-sensitivity ion mobility spectrometry/mass spectrometry using electrodynamic ion funnel interfaces. *Analytical Chemistry* 2005, 77:3330–3339. [PubMed: 15889926]
15. Gillig KJ, Ruotolo BT, Stone EG, Russell DH: An electrostatic focusing ion guide for ion mobility-mass spectrometry. *International Journal of Mass Spectrometry* 2004, 239:43–49.
16. Giles K, Pringle SD, Worthington KR, Little D, Wildgoose JL, Bateman RH: Applications of a travelling wave-based radio-frequency-only stacked ring ion guide. *Rapid Communications in Mass Spectrometry* 2004, 18:2401–2414. [PubMed: 15386629]
17. Dugourd P, Hudgins RR, Clemmer DE, Jarrold MF: High-resolution ion mobility measurements. *Review of Scientific Instruments* 1997, 68:1122–1129.
18. Barnes CAS, Hilderbrand AE, Valentine SJ, Clemmer DE: Resolving isomeric peptide mixtures: A combined HPLC/ion mobility-TOFMS analysis of a 4000-component combinatorial library. *Analytical Chemistry* 2002, 74:26–36. [PubMed: 11795805]
19. Srebalus CA, Li J, Marshall WS, Clemmer DE: Gas-phase separations of electrosprayed peptide libraries. *Anal Chem* 1999, 71:3918–3927. [PubMed: 10500479]
20. Hoadlund-Hyzer CS, Li JW, Clemmer DE: Mobility labeling for parallel CID of ion mixtures. *Analytical Chemistry* 2000, 72:2737–2740. [PubMed: 10905301]
21. Stone E, Gillig KJ, Ruotolo B, Fuhrer K, Gonin M, Schultz A, Russell DH: Surface-induced dissociation on a MALDI-ion mobility-orthogonal time-of-flight mass spectrometer: Sequencing peptides from an “in-solution” protein digest. *Analytical Chemistry* 2001, 73:2233–2238. [PubMed: 11393846]
22. Koeniger SL, Merenbloom SI, Valentine SJ, Jarrold MF, Udseth HR, Smith RD, Clemmer DE: An IMS-IMS analogue of MS-MS. *Anal Chem* 2006, 78:4161–4174. [PubMed: 16771547]
23. El-Baba TJ, Woodall DW, Raab SA, Fuller DR, Laganowsky A, Russell DH, Clemmer DE: Melting Proteins: Evidence for Multiple Stable Structures upon Thermal Denaturation of Native Ubiquitin from Ion Mobility Spectrometry-Mass Spectrometry Measurements. *Journal of the American Chemical Society* 2017, 139:6306–6309. [PubMed: 28427262] **An extensive analysis of the gas-phase conformers produced along the protein melting curve of ubiquitin. Data indicate that two-state folding/unfolding transitions for proteins can contain a number of conformers that are stabilized at different temperatures. The work has tremendous potential to impact the field of protein folding studies. Additionally, as indicated in the manuscript, there is tremendous potential for integrating other techniques for thorough structure characterization.
24. Cryar A, Groves K, Quaglia M: Online Hydrogen-Deuterium Exchange Traveling Wave Ion Mobility Mass Spectrometry (HDX-IM-MS): a Systematic Evaluation. *Journal of the American Society for Mass Spectrometry* 2017, 28:1192–1202. [PubMed: 28374315]
25. Servage KA, Silveira JA, Fort KL, Clemmer DE, Russell DH: Water-Mediated Dimerization of Ubiquitin Ions Captured by Cryogenic Ion Mobility-Mass Spectrometry. *Journal of Physical Chemistry Letters* 2015, 6:4947–4951. [PubMed: 26625010]
26. Zheng XY, Zhang X, Schocker NS, Renslow RS, Orton DJ, Khamsi J, Ashmus RA, Almeida IC, Tang KQ, Costello CE, et al.: Enhancing glycan isomer separations with metal ions and positive and negative polarity ion mobility spectrometry-mass spectrometry analyses. *Analytical and Bioanalytical Chemistry* 2017, 409:467–476. [PubMed: 27604268]
27. Syka JEP, Coon JJ, Schroeder MJ, Shabanowitz J, Hunt DF: Peptide and protein sequence analysis by electron transfer dissociation mass spectrometry. *Proceedings of the National Academy of Sciences of the United States of America* 2004, 101:9528–9533. [PubMed: 15210983]
28. Khakinejad M, Ghassabi Kondalaji S, Tafreshian A, Valentine SJ: Comprehensive Gas-Phase Peptide Ion Structure Studies Using Ion Mobility Techniques: Part 2. Gas-Phase Hydrogen/Deuterium Exchange for Ion Population Estimation. *J Am Soc Mass Spectrom* 2017, 28:960–970. [PubMed: 28315238]

29. Rashid S, Overton S, Mazigh B, Mayer PM: Dual-spray hydrogen/deuterium exchange (HDX) reactions: A new method of probing protein structure. *Rapid Communications in Mass Spectrometry* 2016, 30:1505–1512. [PubMed: 27321838]
30. Uppal SS, Beasley SE, Scian M, Guttman M: Gas-Phase Hydrogen/Deuterium Exchange for Distinguishing Isomeric Carbohydrate Ions. *Analytical Chemistry* 2017, 89:4737–4742. [PubMed: 28304155]
31. Maleki HK AK; Majuta S; Maurer MM; Valentine SJ: Ion mobility spectrometry-mass spectrometry coupled with gas-phase hydrogen/deuterium exchange for metabolomics analyses *J. Am. Soc. Mass Spectrom.* (accepted) 2017.
32. Maleki H, Maurer IM, Ronaghi N, Valentine SJ: Ion Mobility, Hydrogen/Deuterium Exchange, and Isotope Scrambling: Tools to Aid Compound Identification in 'Omics Mixtures. *Analytical Chemistry* 2017, 89:6399–6407. [PubMed: 28505408]
33. Laszlo KJ, Bush MF: Analysis of Native-Like Proteins and Protein Complexes Using Cation to Anion Proton Transfer Reactions (CAPTR). *Journal of the American Society for Mass Spectrometry* 2015, 26:2152–2161. [PubMed: 26323617]
34. Laszlo KJ, Buckner JH, Munger EB, Bush MF: Native-Like and Denatured Cytochrome c Ions Yield Cation-to-Anion Proton Transfer Reaction Products with Similar Collision Cross-Sections. *Journal of the American Society for Mass Spectrometry* 2017, 28:1382–1391. [PubMed: 28224394] *Presents data from novel CAPTR experiments that suggests ion charge should be considered carefully when using restraints for model solution structures. This has implications for obtaining reliable structural information from native MS experiments.
35. Laszlo KJ, Bush MF: Interpreting the Collision Cross Sections of Native-like Protein Ions: Insights from Cation-to-Anion Proton-Transfer Reactions. *Analytical Chemistry* 2017, 89:7607–7614. [PubMed: 28636334]
36. Valentine SJ, Koeniger SL, Clemmer DE: A split-field drift tube for separation and efficient fragmentation of biomolecular ions. *Analytical Chemistry* 2003, 75:6202–6208. [PubMed: 14616002]
37. Koeniger SL, Clemmer DE: Resolution and structural transitions of elongated states of ubiquitin. *Journal of the American Society for Mass Spectrometry* 2007, 18:322–331. [PubMed: 17084091]
38. Eschweiler JD, Rabuck-Gibbons JN, Tian YW, Ruotolo BT: CIUSuite: A Quantitative Analysis Package for Collision Induced Unfolding Measurements of Gas-Phase Protein Ions. *Analytical Chemistry* 2015, 87:11516–11522. [PubMed: 26489593]
39. Eschweiler JD, Martini RM, Ruotolo BT: Chemical Probes and Engineered Constructs Reveal a Detailed Unfolding Mechanism for a Solvent-Free Multidomain Protein. *Journal of the American Chemical Society* 2017, 139:534–540. [PubMed: 27959526]
40. Pisupati K, Tian YW, Okbazghi S, Benet A, Ackermann R, Ford M, Saveliev S, Hosfield CM, Urh M, Carlson E, et al.: A Multidimensional Analytical Comparison of Remicade and the Biosimilar Remsima. *Analytical Chemistry* 2017, 89:4838–4846. [PubMed: 28365979]
41. Allison TM, Reading E, Liko I, Baldwin AJ, Laganowsky A, Robinson CV: Quantifying the stabilizing effects of protein-ligand interactions in the gas phase. *Nature Communications* 2015, 6:10.**Presentation of a powerful approach for studying protein-ligand interactions. Ion complexes are studied with regard to resistance to unfolding. The approach is extremely sensitive. It is also very versatile allowing the study of diverse species such as membrane proteins and their various ligands as well as interactions with lipids.
42. Song Y, Nelp MT, Bandarian V, Wysocki VH: Refining the Structural Model of a Heterohexameric Protein Complex: Surface Induced Dissociation and Ion Mobility Provide Key Connectivity and Topology Information. *Acs Central Science* 2015, 1:477–487. [PubMed: 26744735]
43. Harvey SR, Liu Y, Liu W, Wysocki VH, Laganowsky A: Surface induced dissociation as a tool to study membrane protein complexes. *Chemical Communications* 2017, 53:3106–3109. [PubMed: 28243658]
44. Morrison KA, Bendiak BK, Clowers BH: Enhanced Mixture Separations of Metal Adducted Tetrasaccharides Using Frequency Encoded Ion Mobility Separations and Tandem Mass Spectrometry. *Journal of the American Society for Mass Spectrometry* 2017, 28:664–677. [PubMed: 27796835]

45. Harper B, Neumann EK, Stow SM, May JC, McLean JA, Solouki T: Determination of ion mobility collision cross sections for unresolved isomeric mixtures using tandem mass spectrometry and chemometric deconvolution. *Analytica Chimica Acta* 2016, 939:64–72. [PubMed: 27639144]
46. Morrison KA, Clowers BH: Differential Fragmentation of Mobility–Selected Glycans via Ultraviolet Photodissociation and Ion Mobility–Mass Spectrometry. *Journal of the American Society for Mass Spectrometry* 2017, 28:1236–1241. [PubMed: 28421405] *Presentation of the combination of a novel ion separation and fragmentation strategy that can be used to distinguish glycans. Such an approach holds promise for performing comparative glycomics analyses in biomarker discovery efforts.
47. Cismesia AP, Bailey LS, Bell MR, Tesler LF, Polfer NC: Making Mass Spectrometry See the Light: The Promises and Challenges of Cryogenic Infrared Ion Spectroscopy as a Bioanalytical Technique. *Journal of the American Society for Mass Spectrometry* 2016, 27:757–766. [PubMed: 26975370]
48. Seo J, Hoffmann W, Warnke S, Bowers MT, Pagel K, von Helden G: Retention of Native Protein Structures in the Absence of Solvent: A Coupled Ion Mobility and Spectroscopic Study. *Angewandte Chemie–International Edition* 2016, 55:14173–14176. [PubMed: 27545682]
49. Seo J, Hoffmann W, Warnke S, Huang X, Gewinner S, Schollkopf W, Bowers MT, von Helden G, Pagel K: An infrared spectroscopy approach to follow beta–sheet formation in peptide amyloid assemblies. *Nature Chemistry* 2017, 9:39–44.**Provides strong evidence for the conservation of solution structure into the gas–phase for relatively small proteins. Although the work presents an example of a powerful on–line structure analysis tool, it is important in that it provides further impetus for the use of gas–phase structure analysis tools for the elucidation of solution structure.
50. Voronina L, Masson A, Kamrath M, Schubert F, Clemmer D, Baldauf C, Rizzo TR: Conformations of Prolyl–Peptide Bonds in the Bradykinin 1–5 Fragment in Solution and in the Gas Phase. *Journal of the American Chemical Society* 2016, 138:9224–9233. [PubMed: 27366919]
51. Khanal N, Masellis C, Kamrath MZ, Clemmer DE, Rizzo TR: Glycosaminoglycan Analysis by Cryogenic Messenger–Tagging IR Spectroscopy Combined with IMS–MS. *Analytical Chemistry* 2017, 89:7601–7606. [PubMed: 28636333]
52. Masellis C, Khanal N, Kamrath MZ, Clemmer DE, Rizzo TR: Cryogenic Vibrational Spectroscopy Provides Unique Fingerprints for Glycan Identification. *J Am Soc Mass Spectrom* 2017.
53. Nyron MP, Prentice T, Day J, Kirkpatrick J, Sivalingam GN, Levy G, Haq I, Irving JA, Lomas DA, Christodoulou J, et al.: An integrative approach combining ion mobility mass spectrometry, X–ray crystallography, and nuclear magnetic resonance spectroscopy to study the conformational dynamics of (1)–antitrypsin upon ligand binding. *Protein Science* 2015, 24:1301–1312. [PubMed: 26011795]
54. Gervais D, King D, Kanda P, Foote N, Elliott L, Brown P, Lee NO, Thalassinou K, Pizzey C, Rambo R, et al.: Structural Characterisation of Non–Deamidated Acidic Variants of *Erwinia chrysanthemi* L–asparaginase Using Small–Angle X–ray Scattering and Ion–Mobility Mass Spectrometry. *Pharmaceutical Research* 2015, 32:3636–3648. [PubMed: 26040662]
55. Daly S, Choi CM, Chirot F, MacAleese L, Antoine R, Dugourd P: Action–Self Quenching: Dimer–Induced Fluorescence Quenching of Chromophores as a Probe for Biomolecular Structure. *Analytical Chemistry* 2017, 89:4604–4610. [PubMed: 28351129]
56. Lai AL, Clerico EM, Blackburn ME, Patel NA, Robinson CV, Borbat PP, Freed JH, Gierasch LM: Key features of an Hsp70 chaperone allosteric landscape revealed by ion–mobility native mass spectrometry and double electron–electron resonance. *Journal of Biological Chemistry* 2017, 292:8773–8785. [PubMed: 28428246]
57. Barr JD, Shi LQ, Russell DH, Clemmer DE, Holliday AE: Following a Folding Transition with Capillary Electrophoresis and Ion Mobility Spectrometry. *Analytical Chemistry* 2016, 88:10933–10939. [PubMed: 27809500] *Shows the power of an off–line approach to confirm the results of conformational tracking by IMS–MS analysis. The work also moves toward linking gas– and solution–phase conformers.
58. Mironov GG, Clouthier CM, Akbar A, Keillor JW, Berezovski MV: Simultaneous analysis of enzyme structure and activity by kinetic capillary electrophoresis–MS. *Nature Chemical Biology* 2016, 12:918–+. [PubMed: 27595328]

59. Garabedian A, Butcher D, Lippens JL, Miksovska J, Chapagain PP, Fabris D, Ridgeway ME, Park MA, Fernandez-Lima F: Structures of the kinetically trapped i-motif DNA intermediates. *Physical Chemistry Chemical Physics* 2016, 18:26691–26702. [PubMed: 27711445]
60. Boschmans J, Lemiere F, Sobott F: Analyzing complex mixtures of drug-like molecules: Ion mobility as an adjunct to existing liquid chromatography–(tandem) mass spectrometry methods. *Journal of Chromatography A* 2017, 1490:80–88. [PubMed: 28228233]
61. Baker ES, Livesay EA, Orton DJ, Moore RJ, Danielson WF, Prior DC, Ibrahim YM, LaMarche BL, Mayampurath AM, Schepmoes AA, et al.: An LC–IMS–MS Platform Providing Increased Dynamic Range for High-Throughput Proteomic Studies. *Journal of Proteome Research* 2010, 9:997–1006. [PubMed: 20000344]
62. Liu XY, Valentine SJ, Plasencia MD, Trimpin S, Naylor S, Clemmer DE: Mapping the human plasma proteome by SCX–LC–IMS–MS. *Journal of the American Society for Mass Spectrometry* 2007, 18:1249–1264. [PubMed: 17553692]
63. Lossel P, Brunner AM, Liu F, Leney AC, Yamashita M, Scheltema RA, Heck AJR: Deciphering the Interplay among Multisite Phosphorylation, Interaction Dynamics, and Conformational Transitions in a Tripartite Protein System. *Acs Central Science* 2016, 2:445–455. [PubMed: 27504491]
64. Beveridge R, Migas LG, Payne KAP, Scrutton NS, Leys D, Barran PE: Mass spectrometry locates local and allosteric conformational changes that occur on cofactor binding. *Nature Communications* 2016, 7. **Shows the power of combined techniques in the elucidation of structural details for protein–ligand complexes. Here IMS–MS reveals a shift to smaller conformer types for the holo versus the apo protein. Traditional HDX–MS methods confirm these results by showing structural changes throughout the protein. Such allosteric effects are consistent with the conformational differences distinguished by IMS–MS.
65. Porrini M, Rosu F, Rabin C, Darre L, Gomez H, Orozco M, Gabelica V: Compaction of Duplex Nucleic Acids upon Native Electrospray Mass Spectrometry. *Acs Central Science* 2017, 3:454–461. [PubMed: 28573208]
66. Chouinard CD, Cruzeiro VWD, Beekman CR, Roitberg AE, Yost RA: Investigating Differences in Gas-Phase Conformations of 25-Hydroxyvitamin D3 Sodioted Epimers using Ion Mobility–Mass Spectrometry and Theoretical Modeling. *Journal of the American Society for Mass Spectrometry* 2017, 28:1497–1505. [PubMed: 28417307] *Shows the importance of thorough in-silico modeling to obtain candidate structures. Careful attention to ion structure construction can provide accurate conformer candidates for structures showing only small differences in CCS values.
67. Ghassabi Kondalaji S, Khakinejad M, Tafreshian A, S JV: Comprehensive Peptide Ion Structure Studies Using Ion Mobility Techniques: Part 1. An Advanced Protocol for Molecular Dynamics Simulations and Collision Cross-Section Calculation. *J Am Soc Mass Spectrom* 2017, 28:947–959. [PubMed: 28211014]
68. Mesleh MF, Hunter JM, Shvartsburg AA, Schatz GC, Jarrold MF: Structural information from ion mobility measurements: Effects of the long-range potential (vol 100, pg 16082, 1996). *Journal of Physical Chemistry A* 1997, 101:968–968.
69. Shvartsburg AA, Jarrold MF: An exact hard-spheres scattering model for the mobilities of polyatomic ions. *Chemical Physics Letters* 1996, 261:86–91.
70. Bleiholder C, Wyttenbach T, Bowers MT: A novel projection approximation algorithm for the fast and accurate computation of molecular collision cross sections (I). Method. *International Journal of Mass Spectrometry* 2011, 308:1–10.
71. Larriba-Andaluz C, Hogan CJ: Collision cross section calculations for polyatomic ions considering rotating diatomic/linear gas molecules. *Journal of Chemical Physics* 2014, 141.
72. Marklund EG, Degiacomi MT, Robinson CV, Baldwin AJ, Benesch JLP: Collision Cross Sections for Structural Proteomics. *Structure* 2015, 23:791–799. [PubMed: 25800554]
73. Wyttenbach T, Bleiholder C, Anderson SE, Bowers MT: A new algorithm to characterise the degree of concaveness of a molecular surface relevant in ion mobility spectrometry. *Molecular Physics* 2015, 113:2344–2349.
74. Shrivastav V, Nahin M, Hogan CJ, Larriba-Andaluz C: Benchmark Comparison for a Multi-Processing Ion Mobility Calculator in the Free Molecular Regime. *Journal of the American Society for Mass Spectrometry* 2017, 28:1540–1551. [PubMed: 28477243] *Shows the development of important new tools for calculating CCS values for candidate structures. This work

is important as tools and approaches are being developed to provide a greater number of candidate structures (see references 66 and 67).

75. Young MN, Bleiholder C: Molecular Structures and Momentum Transfer Cross Sections: The Influence of the Analyte Charge Distribution. *Journal of the American Society for Mass Spectrometry* 2017, 28:619–627. [PubMed: 28251573]
76. Landreh M, Marklund EG, Uzdaviny P, Degiacomi MT, Coincon M, Gault J, Gupta K, Liko I, Benesch JLP, Drew D, et al.: Integrating mass spectrometry with MD simulations reveals the role of lipids in Na⁺/H⁺ antiporters. *Nature Communications* 2017, 8.
77. Schiffrin B, Calabrese AN, Devine PWA, Harris SA, Ashcroft AE, Brockwell DJ, Radford SE: Skp is a multivalent chaperone of outer-membrane proteins. *Nature Structural & Molecular Biology* 2016, 23:786–793.
78. Maurer MM, Donohoe GC, Valentine SJ: Advances in ion mobility-mass spectrometry instrumentation and techniques for characterizing structural heterogeneity. *Analyst* 2015, 140:6782–6798. [PubMed: 26114255]
79. Joni S: Cryo-electron Microscopy Analysis of Structurally Heterogeneous Macromolecular Complexes. *Computational and Structural Biotechnology Journal* 2016, 14:385–390. [PubMed: 27800126]
80. Witze A: Research gets increasingly international. Edited by; 2016 vol 2017.]

Highlights

- Application of ion mobility spectrometry - mass spectrometry (IMS-MS) techniques for structure characterization have dramatically increased
- Growth is, in part, attributed to usage with other structure characterization tools
- Both on-line and off-line techniques are employed to enhance/augment IMS-MS data
- The different approaches greatly enrich the structural information afforded by IMS-MS for a diverse array of molecular species

Figure 1.

A) Collision cross section distributions of $[M+6H]^{6+}$, $[M+7H]^{7+}$, and $[M+8H]^{8+}$ ubiquitin ions as a function of solution temperature. Comparisons to the cross sections of the N- and A-states are shown. **B)** Experimental and hypothetical isotopic distributions for peptide fragment ions formed by ETD after precursor ions have undergone HDX inside a drift tube. One conformer type calculated to contribute significantly to the HDX behavior of the ions is shown as the annealed and gas-phase representative structure. **C)** Structural transformations of post-CAPTR protein ions experienced after collisional activation. Panels A, B, C were adapted with permission from figures from references 23, 28, and 34, respectively.

Figure 2.

A) Heat maps depicting the standard deviation in collision-induced unfolding (CIU) for the monoclonal antibodies Remicade (top plot) and Remsima (bottom plot). A small but statistically-significant difference in the CIU of Remsima is highlighted resulting from structural diversity not observed for Remicade. **B)** UVPD fragmentation spectra for mobility-selected glycan ions in a mixture of two isomers. The top two traces represent a high-mobility selection with a comparison to data for the branched form of the glycan. The bottom two traces show data for a low-mobility selection and a comparison with data for the linear glycan isomer. Panels A and B were adapted with permission from figures obtained from references 40 and 44, respectively.

Figure 3.

A) Mass spectral and ion mobility data for acidic variants of non-deamidated L-asparaginase isolated by ion exchange chromatography. The mobility data shows small differences in collision cross section; structural differences in the variants are confirmed by SAXS spectroscopy. **B)** Evolution of different conformer types in the transition of a polyproline peptide from PPI to PPII helix as a function of time. Structural changes were compared with those revealed by ion mobility analysis. Panels A and B were adapted with permission from figures from references 54 and 57, respectively.

Figure 4.

A) Hypothetical structures having matching collision cross sections for the sodiated 25-Hydroxyvitamin D3 Epimers. Lowest energy structures for “open” and “closed” conformer types are shown. **B)** Time required to calculate collision cross sections for trial structures using the MOBCAL and IMoS software. Data are plotted on a log-log plot as a function of size (number of atoms). Panels A and B are adapted with permission from figures from references 66 and 71, respectively.